5 SPECIMEN COLLECTION AND PRESERVATION

This section outlines the use of fixatives and preservatives used in *necropsy* procedures and some specialised collection techniques. Tissue samples should be routinely collected from all organ systems for histopathology if a carcass is fresh (Categories 1–3). Particular attention should be given to collecting and examining samples from areas with gross lesions. Histological samples should include the interface between normal and diseased tissue. Neutral buffered formalin (10% neutral buffered seawater will do the regular buffer is unavailable) is a suitable fixative for general purposes; other solutions may be required for more specialised collection techniques and analyses as discussed in Section 5.2. Crushing, stretching, scraping, or otherwise damaging specimens should be avoided. All samples should be secured in durable, leak-proof containers with appropriate tags as well as internal and external labels.

5.1 Fixatives and Preservatives

5.1.1 10% Neutral Buffered Formalin

The most widely used fixative for wet tissue samples is 10% neutral buffered formalin. For histopathology, samples should be collected from relatively fresh (Categories 1–3) carcasses and cut into small pieces (2 cm x 2 cm x 0.5 cm) and placed in at least 10 volumes of formalin per volume of sample. Larger samples and entire organs may also be preserved in formalin, but these should be cut adequately or infused with formalin using a needle and syringe to insure maximum penetration of formalin into tissue. Do not collect large samples that can not be adequately fixed. Fixation should be complete in 1 to 2 weeks. Samples must be checked routinely during storage to avoid loss of fluid. 10% neutral buffered formalin can be prepared as follows:

Formalin (37% Formaldehyde)	100 ml
Distilled or tap water	900 ml
Disodium hydrogen phosphate Na_2 HPO_4 $2H_2O$	6.5 g
Sodium dihydrogen phosphate Na_2 H_2PO_4 $2H_2O$	4.5 g

Note: Formalin should not be handled without gloves or inhaled. Always use in a well-ventilated area and ensure containers are tightly capped (see Section 3.1).

5.1.2 Bouin’s Solution

Used for preserving gonad samples. Fixation occurs in less than 24 hours. Samples fixed in Bouin’s fluid should be transferred to 10% neutral buffered formalin or 70% ethanol (ethyl alcohol) after 24 hours. Bouin’s fluid can be prepared as follows:

Saturated aqueous picric acid	750 ml
Formalin	250 ml
Glacial Acetic Acid	50 ml

Note: Dry picric acid is explosive. Use extreme care with storage and handling (see Section 3.1).

5.1.3 Ethyl Alcohol EtOH (100%)

Used for preserving nematodes. Formalin (5%) may also be used but is less satisfactory than alcohol.

5.1.4 Acetic Acid

Used for killing certain parasites (see Section 5.2.2).

5.1.5 Dimethyl Sulfoxide (DMSO)

Used to preserve tissue for genetic analyses. Tissue should be preserved immediately in tubes containing 20% DMSO in saturated NaCl solution.

A note on DMSO: DMSO is to be used with caution as it is toxic and mildly flammable. It does not require declaration for transport on aircraft, as it is not toxic or flammable enough to require a United Nations chemical code. However, it is hazardous to your health and should not be ingested. If DMSO comes in contact with your skin it should be immediately washed off with soap and water (see Section 3.1).

5.1.6 Frozen Samples

Samples collected for genetic, contaminants and pesticide analyses should be frozen as soon as possible.

5.2 Specimen Collection Techniques

5.2.1 Ingesta

Stomach contents collected for dietary studies can be preserved in 10% neutral buffered or seawater formalin or in 80% EtOH. About 100 ml of material should be collected from the mouth (if present) and from each region of the digestive tract (including stomach, duodenum, mid-small intestine, caecum, and mid-large intestine) and diluted with an equal volume of preservative. Do not freeze *ingesta* samples – the freezing of seagrass and algal fragments will burst the cell walls and turn the sample mushy, making seagrass identification much more difficult.

5.2.2 Parasites

Parasites need to be killed then relaxed and preserved. Nematodes are best killed by dropping them into glacial acetic acid for one minute, then transferring them to 80% alcohol for storage. Nematodes tend to straighten out when placed in acetic acid, thus making them easier to work with. If acetic acid is not available they may be killed with hot 80% alcohol and then stored in 70–80% alcohol. Formalin (5%) may be used for the preservation of nematodes, but is less satisfactory than alcohol. Flukes need more careful treatment. All flukes may be killed and relaxed using hot (80ºC) water. Larger worms should then be placed on a piece of moist filter paper in a petri dish. This is to render them as flat as possible without compressing them, and is best done with the aid of a dissecting microscope and a pair of needles to move the worms. Another piece of filter paper may then be placed on top of the worms and 10% formalin added, drop-by-drop, until the filter paper is damp. They become sufficiently fixed after about one hour, and can then be removed to a vial of 10% formalin without causing them to curl up again. See Blair (1981) for a description of parasites found in dugongs.

5.2.3 Genetics

The most favoured tissue for genetic analysis from a fresh (Categories 1–3) carcass is gonad, liver or muscle (see Rainey 1981). If the carcass is not fresh, skin (the grey epidermal layer) and/or muscle is the most favoured. Collect a 1 cm x 0.5 cm sample and preserve either frozen

(including blood samples), in 80% EtOH, 20% DMSO in saturated NaCl solution or dried (e.g. salted meat). Tissue preserved in formalin is not favoured.

If a skin biopsy is to be taken from a live animal, it may be surgically removed from the trailing edge of the tail fluke using a sterile scalpel blade or a sterilised leather punch. Only a small amount of tissue is needed. The epidermis of the skin (grey in colour) contains the most DNA. The epidermis (only about 1–2 mm thick) may be scraped off using a sterile scalpel. The white, *fibrous* layer immediately beneath the skin (hyperdermis) is NOT suitable. Once the skin biopsy is obtained, the tissue should be immediately placed into the preservation solution.

5.2.4 Toxic Element and Organohalogen Analysis

Liver, kidney, muscle and blubber tissue samples should be taken as soon as possible. Brain should be sampled if organochlorine pesticide poisoning is suspected. Use a clean stainless steel knife to obtain samples. If possible, wash the knife, then rinse in distilled water and then in ethanol between sampling different tissues. Acetone and hexane rinses are preferred for collection of samples for organohalogen analyses; rinses with dilute nitric acid are preferred for samples collected for elemental analysis. Ensure as far as is practical that samples are not in contact with aerosols from insect repellent, tobacco smoke, exhaust fumes, petrol fumes, hand soap, etc. Samples should be placed individually in glass jars that have been pre-rinsed with acetone, hexane, and dilute nitric acid. If these are not available, samples may be securely wrapped individually in plastic bags or containers such that in the laboratory they can be trimmed and subsampled to provide specimens that have not directly contacted plastic surfaces (see Denton et al. 1980; Geraci and Lounsbury 1993). Samples should be frozen as soon as possible after collecting. Label each sample with date, location and tissue type. Make a note of any unusual field conditions (e.g. recent chemical spills, run-off) that may have an impact on subsequent pollutant levels. Samples that should be collected are detailed below.

Tissue Samples	Area Sampled	Quantity
Blubber (figure 16)	From the outer-most layer of blubber (under the white *fibrous* skin layer) just to one side of the mid-ventral line	300 g (15 cm square, grapefruit size sample)
Liver (figure 16)	From the *caudal* tip of the right lobe of the liver	300 g
Muscle (figure 16)	Collected just to the right of the mid-ventral line (i.e. immediately under the blubber samples	300 g
Kidney (figure 16)	From the *caudal* end of the right kidney	300 g
Milk*	From the teat	As much as possible
Brain**	Half of sagittally cut brain	As much as possible

* Milk may contain organochlorines that can be passed on to juvenile dugongs.
** If organochlorine pesticides are suspected as a cause of mortality, concentrations in brains must be determined. If cholinesterase-inhibiting pesticides are suspected, brain cholinesterase levels should be determined in comparison with controls, and *ingesta* analysed for cholinesterase-inhibiting pesticides.

5.2.5 Urine

Urine can be collected from the urinary bladder of relatively fresh (Categories 1–3) carcasses with a sterile syringe. If the bladder is not distended it may be desirable to slit it to remove the urine with a syringe. Urine can be refrigerated but it should be submitted for culturing or clinical pathology as soon as possible after collecting. Samples can otherwise be frozen for later determination of *osmolality* and other urine values.

5.2.6 Haemolysed Blood

Haemolysed blood from very fresh carcasses (Categories 1–3) can be collected from the heart and stored frozen.

5.2.7 Microbiology

Sampling for microbiological testing may only be possible when a qualified professional (e.g. veterinarian, pathologist and/or trained microbiology technicians) is conducting the *necropsy* and when the tissues are in suitable condition (usually Categories 1–3). Transport swabs should be removed from their sterile wrapping, brushed against the *lesion* or fluid requiring sampling, and replaced in the media tube as swiftly as possible to avoid contamination. The tube should then be labelled, chilled, and submitted to a microbiology laboratory within 72 hours. Separate swabs should be taken from areas where the presence of *pathogen*s is suspected. Specimens may also be collected using rat-toothed forceps and scissors (from sterile packets) and placed in sterile plastic vials on ice. Sampling of the pleural surface of the lungs, bronchi, stomach *mucosa*, perineal fluid, fluid in the pericardial sac, brain surfaces, abscesses, or infected areas is recommended for relatively fresh (Categories 1–3) carcasses.